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THE PRECIPITIN REACTION IN TUBERCULOSIS.*

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IN the serum diagnosis of tuberculosis we are met with difficulties caused either by the slight and varying degree of immunity present, or by complexity of method. On account of the want of constancy which these signs are apt to exhibit, it appears desirable that several should be at our disposal, and that these be as simple and as reliable as possible. At present only one method of serum diagnosis is made use of to any extent for the detection of tuberculosis, Wright's¹ opsonic test, and even its practical usefulness is still under discussion. Agglutination with so-called homogeneous cultures has given good results in the hands of its discoverer, Arloing,² but has not yet succeeded in being adopted more generally. Koch's³ agglutination method has not been able to find its way any better into the clinical laboratories. Therefore Bonome's⁴ initiative in trying to identify and to differentiate various strains of tubercle bacilli by means of the precipitin test met with general interest.

Trying to adapt Bonome's method to clinical exigencies, I have attempted to discover how far the precipitin test in tuberculosis is to be depended upon, and how its technic may be best simplified.

For this purpose I have tested 682 human sera, of which 381 were tuberculous, and 301 normal, at least normal in so far as they were taken from persons in whom tubercle was not suspected.

After many trial experiments in which I tried different tuberculous extracts, and different dilutions of serum, I finally adopted the following procedure:

I took 1 c.c. of Koch's bacillus emulsion and made it up to 20 c.c. with sterile water, shaking and keeping it at 37° for 24 hours. I then separated it into two halves so each of which I added water containing sufficient salt to render the whole isotonic, and in one case sufficient phenol to give a percentage of 0.5. Each part was in this

* Received for publication November 17, 1909.

¹ *Proc. Roy. Soc., Lond.*, 1904, 73, p. 357.

² *Compt. rend. de l'Acad. Sci.*, 1898, 126, p. 1398.

³ *Deut. med. Wchnschr.*, 1901, 27, p. 829.

⁴ *Centralbl. f. Bakt., Orig.*, 1907, 43, p. 391.

way made up to 25 c.c., diluting the original bacillus emulsion to 1:50, both being isotonic, but one having in addition 0.5 per cent phenol. After 12 more hours at 37°, each part was filtered through porcelain.

Altho I kept to this method throughout, I could not discover that bacillus emulsion added immediately to saline solution or to carbolized saline solution, or even where these solutions were added to ground bacteria, and kept at 37° for 36 hours and then filtered, did not give perfectly satisfactory results.

Every week fresh extracts were made.

The greatest difficulty lies in the cleaning of the tubes, which had each to be minutely examined before use. New tubes, I first brushed out with a mixture of soap and fine sand. After washing with water, dilute sulfuric, and distilled water, the tubes were boiled in distilled water. Immediately after use they were washed out with water, and cleaned with a metal rod around which cotton wool had been tightly wrapped, while the tubes were full of water. The narrowest tubes possible were used.

The serum was diluted by means of a capillary pipette, as narrow in bore as possible, from which one drop of serum was allowed to fall into a tube. Twenty drops of 0.5 per cent NaCl were added from the same pipette, and the contents of the tube mixt. Seven drops were taken from this dilution and placed in a second tube, and seven drops into a third. In this way each serum was diluted 1:21, and distributed equally between three tubes. If by chance rather less than one drop of serum was secured, the capillary tube was graduated by means of a mark and the serum diluted in this way, one part of serum to twenty of saline solution, mixt in the first tube, and after that taken up by the pipette and measured in drops if these would divide in three. When all the tubes had been supplied with serum in this way, an equal number of drops of (1) carbolized tuberculous extract, (2) uncarbolized tuberculous extract, and (3) of a solution containing only 0.5 per cent phenol and 0.85 per cent NaCl solution, was dropped into each of these three tubes. The final mixture therefore contained equal parts diluted 1:20 serum, and diluted 1:50 tuberculous extract, or carbolized saline solution.

The contents of the tubes were not allowed to be exposed except when the serum or extract was added.

The serum was rejected if it was not absolutely clear after dilution, or if it was hemolytic or even highly colored.

This dilution of 1:20 is not critical in any way. Other dilutions, if not too weak, act well enough. I chose this dilution because, while not too weak, it yet gave a convenient amount of fluid in the tube for a suspended precipitate to be easily examined.

Stoppered with sterile wads, the tubes were then transferred to the incubator, and left at 37° for 12 hours. I used at first four hours at 37° and 12 at room temperature, but found that this was insufficient. The precipitate after four hours has not in most cases developed, and room temperature is not sufficient to encourage it. On the other hand more than 12 hours is unnecessary. It appears that all sera do not possess an inherent tendency to precipitate. Sera which did not precipitate in 12 hours, did not do so in one or even two weeks.

On one occasion only was I able to obtain a precipitate almost instantaneously when using Fornet and Müller's¹ method of keeping the antigen and the antibody in two separate layers. The majority of

¹ *Ztschr. f. biol. Technik u. Methodik*, 1908, 1, p. 201.

my reactions were too slow in appearing, so that the advantage of this delicate method was lost. No doubt many fine precipitates were missed on this account. Fornet and Krencker¹ however have been able to use this method successfully in tuberculosis.

The serum, after 12 hours' incubation, may remain clear throughout, or it may appear clear but show a slight deposit at the bottom, or in addition to a deposit it may exhibit a more or less intense suspended precipitate.

On account of the extreme slowness of the deposit occasionally, I carried out the test, almost throughout, without knowing whether I was examining tuberculous or normal sera.

Because of the fact that different salts have been shown, especially by the classical experiments of Pauli,² greatly to influence proteid precipitations, I have separated the records of male and female sera, as their salt content, according to the textbook of Simon,³ is very different.

NORMAL SERA.

The normal sera numbered 301, of which 174 were male, and 127 female. Fifty-three sera came from healthy individuals, 248 from persons suffering from other diseases, which included such diseases as carcinoma, diabetes, blood diseases, pneumonia, rheumatic fever, etc.

TABLE 1.
NORMAL SERA.

	No. of Noughts	Percent- age of Noughts	No. of Deposits	Percent- age of Deposits	No. of Suspended Precipitates			Percent- age of Sus- pended Precipi- tates	Tested with
					+	++	+++		
Male sera	121	60.5	28	16	17	7	1	14.3	(1)
	121	60.5	28	16	17	7	1	14.3	(2)
	122	70.1	28	16	17	7	..	13.9	(3)
Female sera ..	85	66.9	30	23.6	11	1	..	9.4	(1)
	86	67.7	29	22.8	11	1	..	9.4	(2)
	90	70.8	27	21.2	9	1	...	7.9	(3)
Total.....	206	68.4	58	19.2	28	8	1	12.3	(1)
	207	68.7	57	18.9	28	8	1	12.3	(2)
	212	70.4	55	18.2	26	8	..	12.2	(3)

Each serum was tested with (1) a 0.5 per cent carbolized tuber-

¹ *Deut. Archiv f. klin. Med.*, 1909, 97, p. 282.

² *Archiv f. Physiol.*, 1899, 78, p. 315.

³ *A Manual of Clinical Diagnosis*, Phila., 1904, p. 30.

culous extract in 0.85 per cent NaCl, (2) an uncarbolized tuberculous extract in 0.85 per cent NaCl, and (3) 0.5 per cent phenol in 0.85 per cent NaCl.

The highest percentage of precipitates was given by carbolized tubercle extract. In a few cases a precipitate, present in (1) and (2), was not present in (3), or in (1) and (3) and not in (2). In almost all cases, however, while the precipitate with carbolic alone

TABLE 2.
TUBERCULOUS SERA.

	No. of Noughts	Percent- age of Noughts	No. of Deposits	Percent- age of Deposits	No. of Suspended Precipitates			Percent- age of Sus- pended Precipi- tates	Tested with
					+	++	+++		
EARLY CASES (110)									
Male (55) {	8	14.5	25	45.4	17	5	..	40	(1)
	9	16.3	24	43.6	17	5	..	40	(2)
	17	30.9	16	29	18	4	..	40	(3)
Female (55) .. {	12	21.8	26	47.3	16	..	1	30.0	(1)
	12	21.8	26	47.3	16	..	1	30.0	(2)
	19	34.5	19	34.5	16	1	..	30.0	(3)
CHRONIC CASES (191)									
Male (103) ... {	12	11.6	27	26.2	40	16	8	62.1	(1)
	15	14.5	25	24.2	39	16	8	61.1	(2)
	18	17.4	24	23.3	39	15	7	59.2	(3)
Female (88) .. {	7	8	24	27.2	34	18	5	59.1	(1)
	8	9	28	31.8	29	18	5	59.1	(2)
	15	17	27	30.6	32	12	2	52.3	(3)
ADVANCED OR ACUTE CASES (80)									
Male (33) {	11	33.3	17	51.5	3	..	2	15.1	(1)
	11	33.3	17	51.5	3	..	2	15.1	(2)
	18	54.5	10	30.3	3	..	2	15.1	(3)
Female (47) .. {	9	19.1	25	53.2	11	1	1	27.6	(1)
	11	23.4	24	51	11	1	..	25.5	(2)
	17	36.1	19	40.4	10	1	..	23.4	(3)

was generally rather less intense, still a precipitate which appeared in the uncarbolized tubercle extract appeared also in the carbolic solution alone which contained no tubercle extract, the serum in all three cases being affected together.

The question of whether these precipitates are specific, caused by the union of precipitinogen and precipitin, is rather intricate. The recent discussion on the specificity of other precipitins shows how undecided this question still is. On the other hand, Naegeli¹ reports that 97 per cent of autopsies carried out by him have demonstrated

¹ Naegeli, quoted from the *Brit. Med. Jour.*, 1909, 2, p. 904.

scars of former tuberculous lesions. Dr. Philip,¹ also, calculates that one-third to one-half of all persons are tuberculous. Bonome² believes that human serum contains normally traces of tubercle precipitin.

I had almost every one of the precipitating normals examined for signs of the disease, but none were to be found. Some of the largest precipitates were given by apparently perfectly healthy persons.

Since Bonome, two interesting papers have been recently published on this subject, by Szaboky,³ and Stoerk.⁴ The cases of Szaboky precipitated the more the farther the disease was advanced. He obtained from seven advanced sera results which were never nil or even weak. This however has not been my experience. A greater percentage of my advanced cases gave no precipitate (26.2 per cent), than of my early cases (18.1 per cent).

The chronic cases, however, yielded remarkable results. Only 9.8 per cent were completely clear, and altogether the precipitates formed were unusually intense.

This was generally but not always the case where the patient was in rather good condition, or where he had suffered from the disease some years previously, and it was apparently arrested.

THE PRECIPITIN REACTION IN PERSONS TREATED WITH TUBERCULIN INJECTIONS.

Twenty-five cases, 20 of which were chronic, were being treated with injections of Beraneck's or Koch's tuberculin, at the time when the serum was taken. The number of cases is perhaps somewhat few to judge from, but the percentage of sera reacting did not show any noticeable increase, altho perhaps there was some increase in intensity.

TABLE 3.
SERA FROM TUBERCULIN TREATED CASES.

	No. of Noughts	Percent- age of Noughts	No. of Deposits	Percent- age of Deposits	No. of Suspended Precipitates			Percent- age of Sus- pended Precipi- tates	Tested with
					—	--	---		
Male.....	2	15.4	3	23	7	1	..	61.5	(1)
Female.....	3	23.3	3	23.3	6	50	(1)

¹ *Brit. Med. Jour.*, 1906, p. 472.

³ *Ztschr. f. Tuberk.*, 1909, 14, p. 169.

² *Centralbl. f. Bakt.*, 1907, 43, p. 391.

⁴ *Wien. klin. Wchnschr.*, 1909, 59, p. 417.

It is interesting that Wassermann and Bruck,¹ when using the complement-deviation test of Bordet and Gengou,² for the presence of tubercle antibody, found it only in cases treated with tuberculin. This observation is supported by the experiments of Citron³ and Lüdke,⁴ but Weil and Straus⁵ and Czastka,⁶ using the same method of complement-deviation, have been successful in demonstrating antibodies in the serum of cases of tubercle which had not been treated with tuberculin.

THE PRECIPITIN REACTION AND THE V. PIRQUET TEST.

Twenty-five cases had been vaccinated after v. Pirquet. Twenty-one of these gave a positive reaction, four were negative.

TABLE 4.

Noughts	Deposits	Suspended Precipitates			v. Pirquet Test
2	7	10	1	1	+ cases
..	3	1	- cases

One normal (?) person, in whom no tubercle could be detected (Dr. M.), was positive to v. Pirquet, and gave a slight deposit in answer to precipitinogen.

It is interesting that all the four cases which had been diagnosed clinically as suffering from tuberculosis, but which were negative to the v. Pirquet test, all gave a positive result to the precipitin test.

COMPARISON OF NORMAL AND TUBERCULOUS SERA.

If the results from all 381 tuberculous sera are put together and compared with the 301 normals, the difference is very striking. The

TABLE 5.
COMPARISON OF NORMAL AND TUBERCULOUS SERA.

Kind of Serum	Percentage of Noughts	Percentage of Deposits	Percentage of Suspended Precipitates	Tested with
Male { Normal.....	69.5	16	14.3	(1)
{ Tuberculous	16.3	36.1	47.6	(1)
Female ... { Normal.....	66.9	23.9	9.4	(1)
{ Tuberculous	14.7	39.4	45.8	(1)
Total { Normal	68.4	19.2	12.3	(1)
{ Tuberculous	15.4	37.8	46.7	(1)

¹ *Deut. med. Wchnschr.*, 1906, 32, p. 449.

² *Compt. rend. de l'Acad. Sci.*, 1903, 137, p. 351.

³ *Berl. klin. Wchnschr.*, 1907, 44, p. 1139.

⁴ *Münch. med. Wchnschr.*, 1908, 55, p. 783.

⁵ *Wien. klin. Wchnschr.*, 1908, 52, p. 1059.

⁶ *Ibid.*, 1908, 21, p. 877.

normal sera gave a negative result in 68.4 per cent of cases, whereas only 15.4 per cent of the tuberculous sera did not precipitate. The number of normal sera giving a suspended precipitate was 12.3, as against 46.7 per cent of the tuberculous sera.

It will be noticed that male and female sera do not differ to any extent.

THE PRECIPITIN TEST AND COMPLEMENT-DEVIATION.

One curious and rather embarrassing fact is apparent in the foregoing records, namely, that where even a considerable precipitate is given by uncarbolyzed extract, or carbolyzed extract, it is also given by carbolic and NaCl alone. This has also been noticed by Stoerk,¹ 60 per cent of whose tuberculous sera behaved in this way.

Moll,² and Welsh and Chapman³ have attempted to prove that the antigen acts as a precipitant without taking any part in the precipitate, but remaining free in the upper fluid. Are we to suppose that the precipitin reaction is of so simple a nature that the precipitinogen can be replaced by such simple chemical agents as phenol in the case of tubercle? Does the presence of precipitin mean simply an inherent tendency toward precipitation? It became necessary to make some attempt to discover whether this precipitation was due to a true precipitin reaction or not.

According to Muir and Martin⁴ and Neisser and Sachs,⁵ complement-deviation is a much more delicate test for the presence of antibodies than is a precipitate reaction.

Complement is not deviated in a serum containing precipitin, or again in one containing precipitinogen. In the presence of both however in suitable proportions, complement is deviated. According to Moll, or Welsh and Chapman, this should be due to the precipitated precipitin; according to Bordet and Gengou, to the union of antigen and antibody.

I accordingly attempted to discover whether complement was deviated at all by these precipitates, and if so, whether the amount of deviation had any relationship to the amount of precipitate.

I took tubes containing mixtures of diluted serum and tubercle

¹ *Wien. klin. Wchnschr.*, 1909, 59, p. 417.

² *Beiträge Chem. Phys.*, 1903, 4, p. 578.

³ *Proc. Roy. Soc., Lond.*, 1906, 78, p. 297.

⁴ *Jour. of Hyg.*, 1906, 6, p. 1181.

⁵ *Berl. klin. Wchnschr.*, 1905, 42, p. 1388.

extract, or carbolic solution, which had been already at 37° for 12 hours. Having noted down the amount of precipitate in each, I then added 0.15 c.c. of human complement, shaking to mix, and placed them again in the incubator at 37° for one hour. After this I added 0.25 c.c. of amboceptor and 1 c.c. of one per cent suspension of ox corpuscles and replaced them in the incubator for three hours with controls.

Deviation of complement did actually occur in certain cases, but had no relation to the amount of precipitate present.

All sera, which I had examined in this way, which had precipitated with the carbolic saline solution alone, did not deviate complement in the slightest.

On the other hand sera, and especially advanced case sera, in which I had not been able to detect the very faintest signs of a precipitate or deposit whatsoever, did deviate the complement completely, or almost so. Altogether 15 advanced case sera, eight of which had yielded no precipitate, were tested in this way and all deviated complement in the cases where tubercle extracts, carbolized or uncarbolicized, had been used. Where carbolic saline alone had been added, however, precipitate or no precipitate, no deviation occurred.

From one point of view this observation may appear against the conception of Moll, and Welsh and Chapman, because the deviation in these unprecipitated advanced case sera was not due to a precipitated antibody. It was also not due to an unprecipitated antibody, because unprecipitated antibody was also present in the corresponding carbolic-alone test, where also no precipitate had occurred.

It appears possible that the precipitin reaction depends upon two processes: (1) the union of antigen and antibody, (2) the precipitation. In these advanced sera, for some reason, the second process may not have taken place.

Altho this explanation agrees best with the classical theory of the relationship between complement-deviation and the precipitin test, analogous and rather striking phenomena have been described by Uhlenhuth,¹ and Muir and Martin,² which appear perhaps to render another view possible. Uhlenhuth observed complement-deviation

¹ *Deut. med. Wchnschr.*, 1906, 32, p. 1244.

² *Jour. of Hyg.*, 1906, 6, p. 1181.

in a serum immunized against the proteid of the lens of the eye, which is incapable of forming a precipitin antibody, or at least of precipitating with its antiserum. Muir and Martin were able to obtain a serum which had been immunized against another closely related species. This serum deviated complement when mixt with the antigen but did not precipitate. It appears at least possible that other bodies exist in an antiserum which can deviate complement. An actual precipitate is evidently not necessary.

However, complement was not deviated in the presence of the antiserum alone.

TABLE 6.

No. of Serum	Kind of Serum	Test with T. B. Extract	Test with Phenol	Hemolysis	Opsonic Count
1.....	tuber.	—	deposit	complete	0.9
2.....	"	—	—precipitate	"	0.3
3.....	"	—	"	"	0.45
4.....	"	—	deposit	"	0.8
5.....	"	—	—precipitate	"	0.66
6.....	"	—	"	"	0.43
7.....	"	—	"	"	0.40
8.....	"	—	—precipitate	"	0.17
9.....	"	—	"	"	0.07
10.....	"	—	"	"	0.06
11.....	"	—	"	"	0.1
12.....	"	—	"	"	0.33
13.....	"	—	"	"	0.2
14.....	"	—precipitate	—	trace	0.1
15.....	"	deposit	—	"	0.23
16.....	"	—	deposit	complete	0.46
17.....	normal	—	clear	"	1.17
18.....	"	deposit	—	partial	0.9
19.....	"	—	deposit	complete	1.16
20.....	"	—	"	"	1.1
NaCl.....					0.23
Control serum 3:17.....					1.07

Putting these facts together, Moll, and Welsh and Chapman find that when antigen and antibody are mixt, the resulting precipitate may contain no precipitinogen. My results go to show that a precipitate may occur when no antigen is present at all.

Muir and Martin, and Uhlenhuth have proved that deviation may occur without precipitation; my results, that in addition, precipitation may occur without deviation.

Muir and Martin,¹ and Heantjens² have showed that opsonin, like complement, is deviated by specific precipitates, formed by the union of antigen and antibody.

¹ *Brit. Med. Jour.*, 1906, 2, p. 1783.

² *Munch. med. Wchnschr.*, 1907, 54, p. 561.

I was anxious to discover whether opsonin behaved also like complement in relation to these non-specific precipitates between phenol and certain sera, that is to say, unaffected by them.

For this purpose I took away 0.05 c.c. of the mixture containing complement and opsonin which had been left in contact with the precipitate for one hour at 37°. The tubes were then supplied with immune serum and corpuscles and put into the incubator at 37° for three hours. Meanwhile an opsonic experiment, done with tubercle bacteria in the method of Wright, was carried out with the small portions of 3:17 diluted serum collected. The results shown in Table 6, p. 95, were obtained.

It appears that altho opsonin is perhaps better absorbed by a slight precipitate if antigen and antibody are both present, it may also be completely deviated by an intense non-specific precipitate, which leaves the complement unaffected.

This interesting fact, that opsonin was absorbed by the non-specific precipitate while complement was not absorbed, is in line with the other differences of behavior between these two bodies which have been pointed out by Fornet and myself.¹

THE NON-SPECIFIC PRECIPITATE.

In describing the precipitate given between phenol and certain sera as non-specific, I do not wish to infer that the precipitate given with tubercle extract was necessarily specific.

The cause of this non-specific precipitate is difficult to explain. Erich Stoerk² states that a reaction may occur in cancer and diabetes. In neither cancer nor diabetes have I found the reaction. In pneumonia and rheumatic fever it was also wanting. He also believes that it may be due to a fatty diet. I have attempted to discover a relationship between diet and precipitation, and have utterly failed.

For example, 40 poor dispensary patients, living at home on a poor diet (24 of them early cases, who precipitate less regularly), gave no precipitate in 20 per cent of cases, which is rather above the average (15.4 per cent). They yielded however a higher percentage

¹ Fornet and Porter, *Centralbl. f. Bakt.*, Abt. I, Orig., 1908-9, 48, p. 461.

² *Wien. klin. Wchnschr.*, 1909, 59, p. 417.

of suspended precipitates more or less marked, 50 per cent in comparison with the average 46.7 per cent.

Again, 14 normal persons, living in a sanatorium and fed upon the same diet as the tuberculous patients, gave no precipitate in 11 cases, a faint deposit in 3 cases. Of 100 tuberculous patients in the same institution, 12 gave no precipitate, while 53 gave a more or less marked suspended precipitate.

I have noticed in several instances that tuberculous patients fed entirely upon milk gave a precipitate while non-tuberculous fed entirely upon milk gave none. The excess of milk which is often characteristic of a consumptive's diet, by altering the calcium content of his serum, cannot very well be credited with encouraging this reaction. Of 30 non-tuberculous persons fed entirely upon milk, 80 per cent were negative to the test, which is above the average for non-tuberculous persons, 68.4 per cent. I could in fact discover no relation between the precipitation and diet.

I have attempted to discover whether the addition of various simple salts, such as calcium, sodium, magnesium salts, chlorides, and sulphates, in a concentration up to $n/4$, could in any way induce a precipitate, which could not be called forth by tuberculous extract or carbolic alone. The only effect which I was able to notice was the natural one of a tendency to keep the precipitate slightly better in suspension, as might have been expected from the slight increase in density of the solution. In no case did a serum, which gave a negative result with tubercle extract or carbolic alone, precipitate when these salts were added, even after a week at 37°, nor did these salts hinder the precipitation as far as I could find. The reason for this non-specific precipitation requires further study.

Whatever the cause however, the fact that so large a percentage of tuberculous sera yield it on the addition of carbolic, is quite sufficient to warrant the use of such a simple and convenient test. The serum requires only to be diluted to say 1:20 by means of a capillary pipette, and added, equal parts, to a 0.5 per cent solution of phenol in 0.85 per cent NaCl solution.

In order to make certain that the serum contains the true antibody, a complement-deviation test, such as that of Wassermann and Bruck, seems advisable. The use of the tubercle extract in a precipitate

test is hardly necessary when the simple and convenient carbolic NaCl solution alone can act almost as well.

I wish to express my thanks to Dr. Macgowan, for immune serum, also to the following physicians for their courtesy in giving me all facilities: Drs. Claude Ker, Guy, Wilson, Selkirk, Maclaren, Krause, Langwill, Boyd, and Ballingall-Watson; also to Dr. Fornet and Dr. Cramer, for their encouragement and advice.